UNIVERSITI PUTRA MALAYSIA

MOLECULAR CHARACTERIZATION OF ESCHERICHIA COLI SEROTYPE 0157:H7 UTILISING CONTEMPORARY PCR TECHNIQUES AND PFGE-GENOTYPING

OOI WAI LING

MOLECULAR CHARACTERIZATION OF *ESCHERICHIA COLI* SEROTYPE 0157:H7 UTILISING CONTEMPORARY PCR TECHNIQUES AND PFGE-GENOTYPING

By

OOI WAI LING

Thesis Submitted in Fulfilment of the Requirements for the Degree of Master of Science in the Faculty of Food Science and Biotechnology University Putra Malaysia

April 2000

DEDICATIONS

My beloved parents, brothers, sisters, relatives and friends. Also to those who have taught me and still continue in teaching me of different angles of life. Also to those who have sincerely provide invaluable assistance, guidance, advice, moral support and encouragement through out the whole project including the writing out part.

WAI LING 2000

Abstract of thesis presented to the Senate of University Putra Malaysia in fulfilment of the requirements for the degree of Master of Science.

MOLECULAR CHARACTERIZATION OF *ESCHERICHIA COLI* SEROTYPE 0157:H7 UTILISING CONTEMPORARY PCR TECHNIQUES AND PFGE-GENOTYPING

By

OOI WAI LING

April 2000

Chairman: **Dr. Son Radu**

Faculty: **Food Science and Biotechnology**

A total of 28 strains of *Escherichia coli* 0157:H7 which were originally isolated from beef and chicken samples from various locations in Malaysia were examined and further characterized by various molecular techniques. These techniques include the plasmid profiling, antibiotic resistance, multiplex PCR, RAPD pattern, PFGE, and also ERIC fingerprinting. All the strains utilized in this study were found to exhibit a multiple antibiotics resistance pattern to the fourteen antibiotics [bacitracin (100%), penicillin G (100%), sulphafurazole (82%), ampicillin (61%), cephalothin (57%), carbenicillin (50%), ceftazidime (39%), erythromycin (32%), streptomycin (18%), nalidixic acid (14%), chloramphenicol (11%), kanamycin (7%), latamoxef (7%), and tetracycline (7%)] used. The plasmid profile obtained ranged in sizes from 3.23 MDa to 60 MDa. Development of a PCR identification assay for *Escherichia coli* 0157:H7 is based on the isolation of species-specific DNA. Two types of specific primer encoding the Shiga-Like Toxin gene, the *SLT*II (584 bp) gene and *SLT*I (348 bp) were utilized in the multiplex PCR assay. Analysis carried out

demonstrated that *Escherichia coli* 0157:H7 strains were positive for the presence of single *SLT*II gene (17.9 %) or both the *SLT*I and *SLT*II genes (82.1 %). Three 50% G+C contents 10-mer random primers, the Gen 1-50-01 (5′-GTGCAATGAG-3′), Gen 1-50-06 (5′-AGGTTCTAGC-3′), and Gen 1-50-09 (5′-AGAAGCGATG-3′) were chosen after screening through ten random primers. In PFGE technique carried out, two kinds of restriction enzymes, the *Spe*I (5′-A↓CTAGT-3′) and *Xba*I (5′-T↓CTAGA-3′) were used to check for the *in-situ* DNA digestion pattern due to their inherit advantages of the short sequence of these enzymes. Both the RAPD polymorphism pattern and the PFGE profile obtained showed a significant discriminatory fingerprinting among the 28 isolates under studied. A respective dendrogram was constructed from the binary data matrix obtained from the RAPD, PFGE and ERIC fingerprints to compare the diversity relationship among the 28 isolates. All the dendrograms were constructed utilising the RAPDistance software package based on the data retrieved from the presence or absence of banding pattern. ERIC genotyping technique was used as an additional molecular typing method to further assists in the molecular characterization of the *Escherichia coli* 0157:H7. Interestingly, all the three molecular techniques of RAPD, PFGE, and ERIC genotyping showed a significant correlation whereby the first 14 and the second 14 isolates of *Escherichia coli* 0157:H7 used in this study showed a closer relationship in the respective cluster groups as shown in the constructed dendrograms. From the overall results obtained both the RAPD and PFGE analysis showed greater discriminatory power compared to the other phenotypic and molecular characterization techniques used in this study. Our results demonstrate that the plasmid profiling, multiplex PCR, RAPD-PCR fingerprinting, PFGE and ERIC profiling methods are able to act as one of the most appropriate and suitable analysis

tools for a rapid and reliable molecular typing and identification of *Escherichia coli* 0157:H7.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
Sebagai memenuhi keperluan untuk ijazah Master Sains.

PENCIRIAN SECARA MOLEKUL *ESCHERICHIA COLI* SEROTIP 0157:H7 MENGGUNAKAN KAEDAH-KAEDAH PCR DAN GENOTIPIK PFGE

Oleh

OOI WAI LING

April 2000

Pengerusi: **Dr. Son Radu**

Fakulti: **Sains Makanan dan Bioteknologi**

Sejumlah dua puluh lapan strain *Escherichia coli* 0157:H7 yang diisolasikan pada asalnya dari sampel daging lembu dan ayam yang diperolehi daripada beberapa lokasi di Malaysia telah diperiksa dan dicirikan selanjutnya dengan menggunakan pelbagai teknik molekul. Teknik-teknik ini merangkumi penganalisaan profil plasmid, ujian ketahanan terhadap antibiotik, multiplek PCR, fingerprint RAPD, PFGE, dan juga ERIC. Kesemua strain yang digunakan di dalam kajian ini didapati memaparkan keperbagaian corak kerintangan antibiotik terhadap empat belas antibiotik [bacitracin (100%), penicillin G (100%), sulphafurazole (82%), ampicillin (61%), cephalothin (57%), carbenicillin (50%), ceftazidime (39%), erythromycin (32%), streptomycin (18%), nalidixic acid (14%), chloramphenicol (11%), kanamycin (7%), latamoxef (7%), dan tetracycline (7%)] yang digunakan. Profil plasmid yang diperolehi menunjukkan julat saiz antara 3.23 MDa ke 60 MDa. Perkembangan assai identifikasi PCR untuk *Escherichia coli* 0157:H7 adalah berasaskan isolasi DNA yang spesifik kepada spesis. Dua jenis primer spesifik yang mengkodkan gen "Shiga-Like Toxin", iaitu gen *SLT*II (584 bp) dan gen *SLT*I (348 bp) telah digunakan di dalam assai

multiplek PCR. Analisis yang dijalankan mendapati bahawa *Escherichia coli* 0157:H7 menunjukkan sama ada kehadiran gen *SLT*II secara tunggal (17.9%) atau kedua-duanya sekali (82.1%). Tiga primer rawak (10-mer) yang mengandungi kandungan G+C sebanyak 50%, iaitu primer Gen 1-50-01 (5′-GTGCAATGAG-3′), Gen 1-50-06 (5′-AGGTTCTAGC-3′), dan Gen 1-50-09 (5′-AGAAGCGATG-3′) telah dipilih selepas ujian saringan dijalankan ke atas 10 primer rawak. Di dalam teknik PFGE yang dijalankan, dua jenis enzim pemotong, iaitu enzim *Spe*I (5′-A↓CTAGT-3′) dan *Xba*I (5′-T↓CTAGA-3′) telah digunakan berdasarkan kebaikan tersendiri "sequence" pendek enzim-enzim ini bagi penghasilan corak penghadaman DNA pada setempat (*in-situ*). Kedua-dua corak polimorfik RAPD dan profil PFGE yang diperolehi menunjukkan diskriminasi fingerprint secara signifikan ke atas 28 isolat yang dikaji. Dendrogram yang berasingan telah dibina dari maklumat data matrik binari yang didapati daripada fingerprint RAPD, PFGE dan ERIC bagi membandingkan hubungan diversiti antara 28 isolat tersebut. Kesemua dendrogram yang dihasilkan adalah dibina menggunakan pakej cakera liut RAPDistance yang berasaskan data yang didapati dari corak kehadiran atau ketidakhadiran "band". Pengenotipikan teknik ERIC turut dilakukan sebagai satu tambahan penganalisaan secara molekul bagi membantu pencirian lanjutan secara molekul *Escherichia coli* 0157:H7. Profil genotipik yang terhasil daripada teknik-teknik molekul RAPD, PFGE dan ERIC, memaparkan suatu korelasi signifikan di antara isolat *Escherichia coli* 0157:H7 yang digunakan. 14 isolat yang pertama dan kedua masing-masing memaparkan hubungan rapat di dalam kumpulan kelas yang sama seperti yang ditunjukkan dalam dendrogram yang dibina. Dari keseluruhan keputusan yang diperolehi, kedua-dua teknik penganalisaan RAPD dan PFGE didapati menunjukkan kuasa diskriminasi yang jauh lebih tinggi berbanding kaedah pencirian molekul dan fenotipik yang lain. Keputusan yang

didapati juga menunjukkan bahawa kaedah profil plasmid, multiplek PCR, fingerprint RAPD-PCR, PFGE dan ERIC berupaya digunakan sebagai salah satu alat penganalisaan yang cepat, berkesan dan bersesuaian bagi pencirian molekul dan identifikasi *Escherichia coli* 0157:H7.

ACKNOWLEDGEMENTS

I would particularly like to express my deepest gratitude to my supervisor Dr. Son Radu for his continuous support, patience, invaluable advice, and guidance through out the course of this project. My deepest gratitude to my co-supervisors, Professor Dr. Gulam Rusul Rahmat Ali and Professor Dr. Mohd Ismail Abdul Karim. Without their persistent assistance and exceptional generosity, this work would not have been possible and meet the requirement of the course.

Special thanks are extended to all the postgraduate students of Food Borne Pathogen and Molecular Subtyping Group for their continuous support and assistance through out the whole project.

Heart felt thanks to those helpful people from the Department of Biotechnology and Food Science, and Graduate School (GSO) who have assisted and supported me either directly or indirectly through out the whole duration of my project and the writing up for the final thesis.

Last but not least of all, a special note of thanks to my family and friends, who share much of my joy and sorrow. Words just cannot express my gratitude for their love, concern, support and patience that have sustained me throughout the whole course of my post-graduate studies.

I certify that an Examination Committee met on 26th April, 2000 to conduct the final examination of Ooi Wai Ling on her Master thesis entitled " Molecular Characterization of *Escherichia coli* Serotype 0157:H7 Utilising Contemporary PCR Techniques and PFGE-Genotyping" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examniation Committees are as follows:

Hirzun Mohd. Yusof, Ph.D.
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Chairman)

Son Radu, Ph.D.
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)

Gulam Rusul Rahmat Ali, Ph.D.
Professor
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)

Mohd. Ismail Abdul Karim, Ph.D.
Professor
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)

MOHD GHAZALI MOHAYIDIN. Ph.D.
Professor / Deputy Dean of Graduate School,
Universiti Putra Malaysia.

Date: 31 MAY 2000

This thesis submitted to the Senate of Universiti Putra Malaysia and was accepted as fulfilment of the requirements for the degree of Master Science.

KAMIS AWANG, Ph.D.
Associate Professor,
Dean of Graduate School
Universiti Putra Malaysia

Date: 13 JUL 2000

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

(OOI WAI LING)

Date: 31 MAY 2000

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

Abbreviations

A	adenine or adenosine
AP-PCR	arbitrarily primed-polymerase chain reaction
ATCC	American type culture collection
ATP	adenosine triphosphate
Amp	ampicillin
B	bacitracin
bp	basepair
BSA	bovine serum albumin
C	chloramphenicol
Car	carbenicillin
Cf	cephalothin
Caz	ceftazidime
ccc	covalently closed circular
cm	centimetre
Da	dalton (the unit of molecular mass)
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytosine triphosphate
dGTP	Deoxyguanosine triphosphate
dH_2O	Distilled water
DNA	deoxyribonucleic acid
dTTP	Deoxythymidine triphophate
E	erythromycin

E.coli	*Escherichia coli*
e.g.	For example
EDTA	Ethylenediamine tetraacetic acid
EtBr	ethidium bromide
g	Gram
g	Gravity
G	Guanine
GTP	Guanosine triphosphate
H_2O	Water
HCl	Hydrochloric acid
i.e.	that is
ID	Identification number
K	kanamycin
kb	Kilobase pair (number of bases in thousands)
Kda	kiloDalton
kg	kilogram
LB	Luria-Bertani
M	Molar, or molarity, moles of solute per liter of solution
mA	milliamphere
MDa	megadalton
mg	milligram
MHA	Muller Hinton agar
min	Minutes
ml	Milliliter
mm	millimeter

mM	Millimolar
Mox	latamoxef
µg	Microgram
µl	Microlitre
mol	mole
Na	nalidixic acid
NaCl	Sodium chloride
NaOH	Sodium hydroxide
ng	Nanogram
P	penicillin
%	Percent
R	resistant
RAPD	Random amplified polymorphic DNA
RNA	ribonucleic acid
RNase	ribonuclease
rpm	revolution per minute
S	sensitive
S	streptomycin
Sf	sulphafurazole
sdH_2O	Sterile distilled water
SDS	sodium dodecyl sulphate
TAE	Tris acetate EDTA electrophoresis buffer
Taq	Thermus aquaticus DNA (polymerase)
TBE	Tris borate EDTA electrophoresis buffer
TE	tris-EDTA

Te	tetracycline
Tris	tris (hydroxymethyl)methylamine
UV	ultraviolet
V	Volts

CHAPTER I

GENERAL INTRODUCTION

In the year 1885, a German microbiologist, Theobald Escherich had discovered a new bacterium, named *Escherichia coli* (Escherich, 1885). This bacterium is generally regarded as benign commensal in humans intestine and was first thought to belong to the normal microflora of man and animals (Sojka, 1965). However, this view had such change due to various incidences of disease-related strains of *E.coli* had been found.

Based on the distinct clinical manifestations, virulent determinants, and other genetic properties, these strains of *E.coli* are further classified in a later period of time into different groups of enteropathogenic *E.coli* (EPEC), enterotoxigenic *E.coli* (ETEC), enterohaemorrhagic *E.coli* (EHEC), enteroinvasive *E.coli* (EIEC), entero-aggregative *E.coli* (EAEC), and enteroverocytotoxic *E.coli* (EVEC).

Most *Escherichia coli* strains are harmless commensals in the humans' gut. Some strains can cause severe food-borne diseases especially those caused by the enterohaemorrhagic *Escherichia coli* (EHEC) (Levine, 1987; Gannon *et al.*, 1992; Padhye and Doyle, 1992). Among the various foodstuffs reported to harbor *E.coli*, raw milk and pasteurized milk have been implicated as source and vehicle of *E.coli* infection that resulted in cases of severe human diarrhea illness (Borczky *et al.*, 1987; Griffin and Tauxe, 1991; Feng, 1995). The risk groups for this bacteria related illness are children and immunocompromised patients. Consumption of contaminated milk